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ERYTHROPOIETIC STEM CELL RECOVERY IN IRRADIATED POLYCYTHEMIC DOGS

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ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

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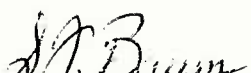

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FOREWORD
(Nontechnical summary)

Before a red cell moves into the circulation to assume its function as an oxygen carrier for tissue and cellular metabolism, it must go through several maturation stages in the bone marrow. The most primitive or multipotential stem cell is first differentiated into a cell which is specific for red cell productivity. During this committed stage this cell is sensitive to the hormone erythropoietin which initiates its capability to synthesize hemoglobin and therefore ultimately to produce mature red cells.

Since the earlier more primitive cells reproduce frequently, they are highly radiosensitive. However, in general, the erythropoietic system has the capability for cellular self-renewal and therefore it can repair itself after exposure to sublethal radiation. This system is controlled by a feedback mechanism. Sensitive cells in the kidney detect the intracellular partial oxygen pressure which depends upon the oxygen carried by the red cells. When the partial pressure is reduced, indicating a low level of oxygen carried by the red cells, erythropoietin is produced. This hormone stimulates the production of red cells from the committed stage.

In a previous study, the capability for postirradiation recovery of the red cell producing system was measured. It was found to recover in an oscillatory pattern during the early weeks after exposure.

The present experiment was designed to investigate the origin of this oscillatory recovery response. Dogs were hypertransfused with red cells from donor animals which inhibited new red cell formation by the erythropoietic system. The capability

of the stem cells to respond to measured quantities of erythropoietin, as indicated by ^{59}Fe incorporation into newly formed cells, was used as an assessment of the erythrocytic stem cell compartment size in normal dogs as well as in animals subjected to a sublethal exposure of 150 rads of mixed gamma-neutron radiation. It was observed that the origin of the postirradiation oscillations in red cell recovery is within the stem cell compartment. It is proposed that, in the main, these oscillations are caused by differences between the differentiation rate of multipotential uncommitted stem cells and those committed for red cell production.

ABSTRACT

The response of erythropoietic stem cells in postirradiated polycythemic dogs to 300 units of administered exogenous erythropoietin was measured. One week prior to irradiation the dogs were made polycythemic by transfused allogeneic erythrocytes. The dogs were subjected to 150 rads of mixed gamma-neutron radiation. On the day of irradiation or on 10 different days during the first 3 weeks postirradiation, erythropoietin was administered and the ^{59}Fe incorporation in response to this stimulation was measured. Recovery of the erythropoietic stem cells occurred in an oscillatory manner. In general, this was ascribed to a possible slow rate of differentiation of erythropoietin responsive cells from multipotential stem cells and a more rapid rate of proliferation of the former cells into subsequent erythrocytic progeny. As the number of divisions resulting from each cell is limited, recovery in number of cells is halted or even decreased until more cells from the slower moving multipotential stem cell compartment are released. Contributions to this oscillatory recovery pattern by chalones or the competition of related blood cell lines for the common stem cells, however, have not been excluded.

I. INTRODUCTION

Recently it was reported[2] that postirradiation erythropoietic recovery in dogs occurs in an oscillatory pattern. It was suggested that these oscillations were the result of cell population adjustments within the stem cell compartment and probably were not due to fluctuations in the stimulation for the release of cells to committed cell lines. This seems to be further supported by reports which indicate that erythropoietin levels apparently do not change in sublethally irradiated rats or dogs.[9,13,14]

In order to obtain more direct evidence for the origin of the postirradiation oscillatory erythropoietic recovery, an experiment was designed utilizing the polycythemic dog as the test animal. Since erythropoiesis is greatly reduced in this animal, the response of its erythropoietin responsive cells to a measured quantity of administered erythropoietin is a measure of its capability for erythropoiesis at the time of the hormone injection.

II. METHODS

Healthy, purebred, AKC registrable male beagles, 1 to 2 years old, from the colony of the Armed Forces Radiobiology Research Institute (AFRRI) were used in this study. They were under a veterinarian's care for the prevention of parasite infestation and were immunized against distemper, hepatitis and rabies.

During the experiment the dogs were housed individually in stainless steel cages in temperature-controlled rooms. They were fed kibbled laboratory dog food supplemented once a week with high protein canned meat ration. Water was provided ad libitum.

A total of 102 dogs were utilized in this study; 18 were employed to determine the optimal concentration of erythropoietin* necessary to stimulate erythropoiesis in unirradiated polycythemic dogs, and 84 were used in the main experiment. Of the latter group, 77 were subjected to 150 rads of mixed gamma-neutron radiation while 7 served as nonirradiated controls.

The absence of data on dogs in the literature made it necessary to determine experimentally the optimum concentration of erythropoietin to stimulate erythropoiesis in polycythemic dogs. This was particularly important in light of recent work by Byron[5] who demonstrated increasing fluctuation in erythropoietic responses when submaximal hormone doses were employed. In our hands, 6 units of erythropoietin stimulated maximal erythropoietic responses in polycythemic rats. On the basis of a fiftyfold plasma volume increase for dogs, the a priori estimate was approximately 300 units of erythropoietin.

Since sheep Erythropoietin A, Step 1, which can be used safely in rodents, was toxic when administered in larger concentrations to dogs, the more purified albeit more expensive Step 3 preparation was employed. However, only limited quantities of this preparation were available; therefore the titration studies were carefully conducted with a small number of animals.

Table I indicates that whereas 100 units of erythropoietin have no effect, the administration of 200 units induces a ^{59}Fe uptake of 59.5 percent and 300 units could well have been maximal in stimulating erythropoiesis. Additional tests as delineated

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* Erythropoietin, Step 3 (sheep), obtained from Connaught Medical Research Laboratories, Toronto, Canada

in Table II seem to confirm this. Apparently no further erythrocytic cell production is initiated with hormone doses above 300 units.

Table I. Radioiron Uptake in Polycythemic Dogs in Response to Administered Exogenous Erythropoietin

Number of dogs	Erythropoietin (units)	^{59}Fe Uptake (percent of injected dose)
3	0 (saline)	19.7 ± 2.9*
3	100	22.7 ± 5.1
3	200	59.5 ± 3.9
3	300	64.4 ± 6.1

* Standard error

Table II. Radioiron Uptake in Polycythemic Dogs in Response to Administered Exogenous Erythropoietin

Number of dogs	Erythropoietin (units)	^{59}Fe Uptake (percent of injected dose)
3	0 (saline)	26.0 ± 2.0*
1	300	61.5
7†	300	61.5 ± 8.4
1	500	56.2
1	700	49.2

* Standard error
† Control dogs from the main experiment

The dogs were subjected to mixed gamma-neutron radiation from the AFRRI-TRIGA reactor. The absorbed dose (150 rads) is at the midline of the animal. The tissue kerma rate, free-in-air, was 20 rads/minute and the ratio of neutron kerma to gamma ray kerma, free-in-air, was 0.67. This ratio was not measured in tissue

where it was undoubtedly somewhat reduced. The effective gamma energy was between 1 and 2 MeV.

One week prior to irradiation, all dogs were made polycythemic by three transfusions, one every other day, of approximately 250 ml of packed allogeneic erythrocytes. To assure the maintenance of polycythemia, dogs administered erythropoietin beyond the 9th day postirradiation received a fourth transfusion of packed cells. On the day of irradiation or on postexposure days 1, 3, 5, 7, 9, 11, 12, 15, 18 or 21, groups consisting of seven different polycythemic dogs received intravenously 300 units of erythropoietin. A similar hormone dose was administered to seven unirradiated animals at the approximate midpoint of the experiment (day 12). Two days after the erythropoietin injection, the animals were injected via the left jugular vein with 1 ml of a sodium citrate-buffered $FeCl_3$ solution containing 10 μCi of ^{59}Fe in 0.1 μg of total iron. A blood sample was obtained 7 days after the isotope injection and the ^{59}Fe incorporation was determined. The methodology for the determination of the radioactivity and the radioiron uptake was described previously.[2] The hematocrit, measured by the standard microhematocrit method, was determined for each dog on the day of erythropoietin and ^{59}Fe injection as well as on the day of blood sampling for the iron uptake determination.

III. RESULTS

Figure 1 clearly indicates that the hematocrits of the dogs were significantly elevated throughout the 23 days of the present experiment and that all animals were polycythemic. As may be seen in Tables I and II, iron uptake and consequently erythropoiesis is greatly reduced in polycythemic dogs not receiving exogenous

erythropoietin. The ^{59}Fe uptake in the seven control animals was 61.5 percent of the injected activity which was very close to most values obtained in unirradiated dogs receiving erythropoietin in quantities of at least 200 units.

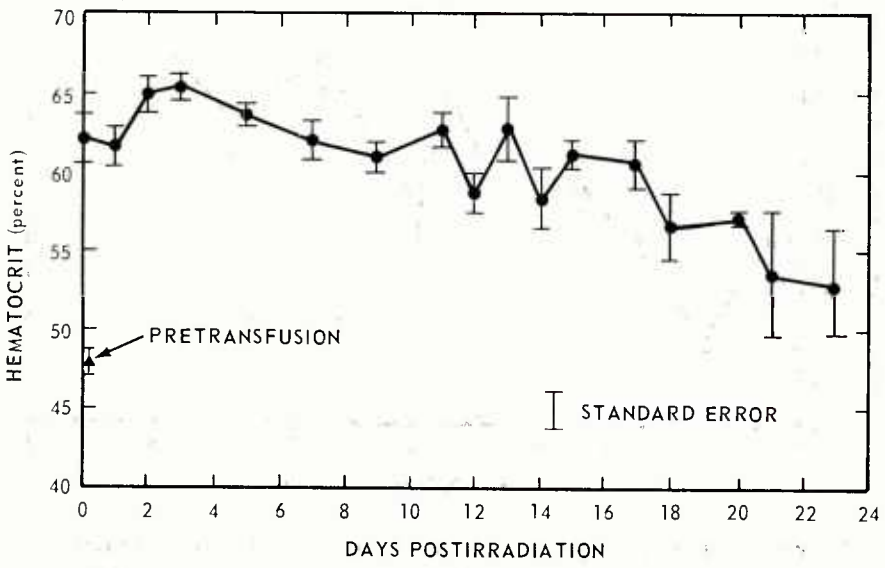

Figure 1. Hematocrits for polycythemic dogs subjected to 150 rads mixed gamma-neutron radiation

Iron incorporation in response to the administration of 300 units of erythropoietin immediately after irradiation (day 0) was reduced to approximately 17 percent of values obtained from unirradiated polycythemic dogs (Figure 2). This was followed by an apparent abortive rise 1 day later with about twice the uptake values. A second low value was measured on the 3rd day, followed by increases reaching approximately 50 percent of control values on day 7 postirradiation. No further increase is noted on the 9th day, followed by the third erythropoietic recovery to approximately 70 percent of control values on the 11th day. Thereafter, another depression is seen on the 12th day, a fourth recovery peak (approximately 76 percent) on the 15th day, one more

return to possible lower ^{59}Fe uptake values on the 18th day and continued recovery beyond that time.

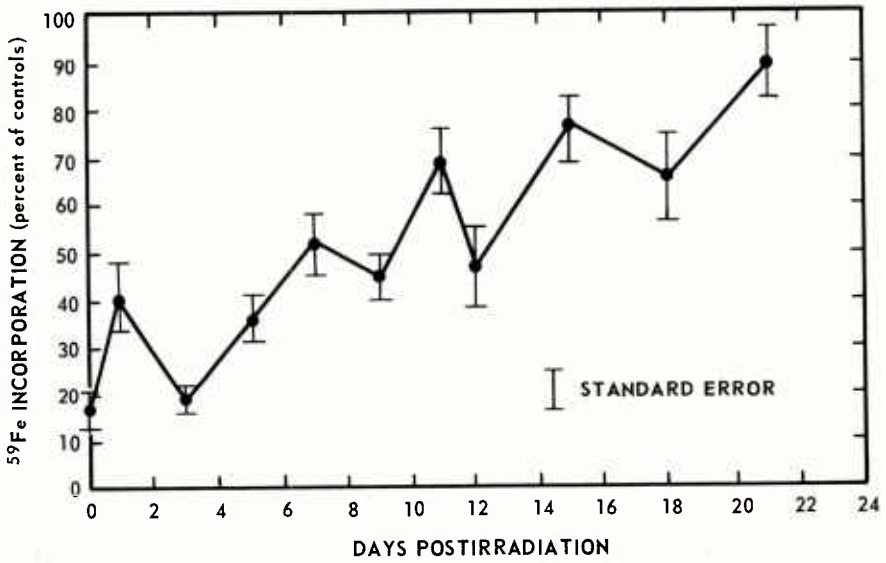

Figure 2. Erythrocytic stem cell recovery in polycythemic dogs exposed to 150 rads mixed gamma-neutron radiation

IV. DISCUSSION

Earlier reports[6,7] have demonstrated the validity of the assumption that the erythropoietic response in polycythemic animals to exogenous erythropoietin is a measure of their erythrocytic stem cell compartment size. This was further confirmed in a more recent experiment.[11] The data of the present study appear to establish that the origin of the postirradiation oscillatory erythropoietic recovery pattern in dogs is in the erythrocytic stem cell compartment.

It is of interest to compare the postirradiation recovery curve of the polycythemic animals in the present study with that of dogs with normal red cell mass published earlier.[2] First, it appears that the radiation effect upon the erythropoietic stem cells

as seen in the present study is much more severe when compared with that upon the total red cell precursor system seen previously (Figure 3). Iron uptake was reduced to 17 percent of control values in the dogs of the present study, while only to 84 percent in those of the previous study. Thereafter, it appears that recovery peaks for stem cells precede those for the total precursor system. The maxima for the first three consecutive peaks appear to occur about 3 to 4 days earlier in the polycythemic dogs. One might suggest the possibility that it takes approximately a 3- to 4-day period in dogs for released stem cells to convert to hemoglobin synthesizing cells. The higher iron incorporation values observed in nonpolycythemic irradiated dogs and reported in the previously published study[2] represent the capability of the precursor system for amplifying cellular production in animals where erythrocytic stem cells

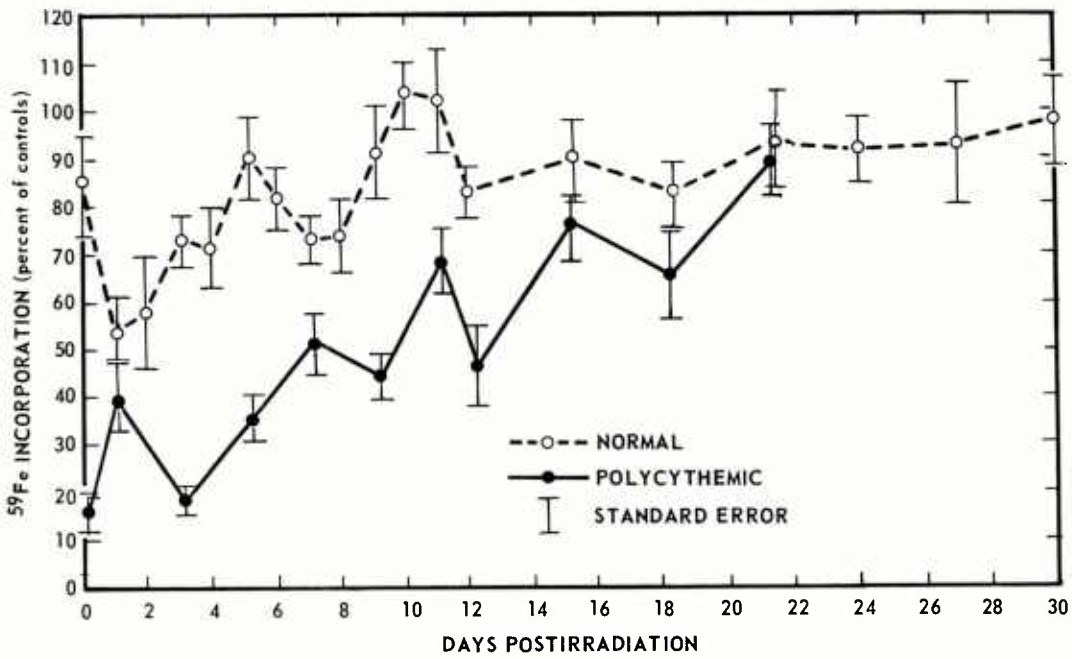

Figure 3. Erythropoiesis in normal and in polycythemic dogs exposed to 150 rads mixed gamma-neutron radiation

are released continuously due to stimulation by endogenous erythropoietin. It is assumed that the iron uptake values measured in the polycythemic dogs of the present experiment represent only the maximum number of erythropoietic stem cells capable of release in response to the administration of exogenous erythropoietin and their subsequent progeny.

Having ascertained that the origin of the postirradiation erythropoietic oscillations is within the bone marrow population, attempts to explain the pathophysiological basis are indicated. Morley and Stohlman[10] have presented evidence that normal erythropoiesis in dogs may occur in an oscillatory manner. These oscillations may be initiated by an inhibitor or chalone, the concentration of which controls stem cell compartment size.[8,12] However, particularly as the data of the present experiment indicate, the number of erythropoietic stem cells is drastically reduced after irradiation which in turn should have decreased the concentration of chalone. Oscillatory production of cells could possibly have occurred only if the inhibitions were initiated by the chalone concentration per stem cell.

Results of the present experiment appear not to support the contention by Bullough and Rytömaa[4] that chalone might be produced by circulating erythrocytes. In the postirradiated dog, erythropoiesis starts before a significant decline in mature eythrocytes is noted. This would be at a time when chalone concentration should still have been at a relatively high level if produced by erythrocytes. Furthermore, the concentration of peripheral erythrocytes was maintained artificially at an above normal level throughout this study.

Although chalones might control the normal turnover of stem cells, it is not entirely clear how or if they act during the early postirradiation days. Alternatively, the data of the present study suggest the following mechanisms. Within hours after irradiation, feedback stimulations from the now decimated committed erythropoietin responsive cell (ERC) population induce the release of cells from the multipotential stem cell compartment. The latter is of course extremely limited since the number of these cells was also reduced by radiation. Once released, the ERC's proliferate rapidly as indicated by the first rise (day 1) in the present study. However, there appears to be some constraint operating which permits these cells in polycythemic animals to multiply up to a certain number and not beyond.[11] Since differentiation from the multipotential stem cells to the ERC's apparently occurs at a slower pace[3] than the proliferation rate of the latter, further increase in size is halted. Indeed, if the final ERC's could only respond to erythropoietin within a relatively short period of time, this specific cell population in polycythemic animals would decrease before sufficient additional differentiation from the stem cells has occurred. Evidence for this was apparently obtained in a study using polycythemic mice.[11] In the normal nonpolycythemic animal, decreases in cellular populations would simply occur by the rapid release of ERC's into the erythrocytic cell lines as stimulated by endogenous erythropoietin and a slower rate of replacement from the multipotential stem cells. As more stem cells differentiate into ERC's, the next recovery cycle begins. In this way, differences in the rate of cell differentiation from the primitive multipotential stem cells to the committed ERC's could quite possibly have been responsible for the

oscillations in the stem cell compartment and consequently in red cell production observed in the present experiment.

However, this does not exclude possible contributions by chalone or the competition of related cell lines (i.e., leukocytes) for the common stem cell. A so-called abortive rise in leukocytic response has been reported in the dog during the 2nd week postirradiation.[1] The unequal oscillations observed in the present study suggest greater complications as the postirradiation recovery time progresses than could be explained by any one single process.

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